PATENT SPECIFICATION

NO DRAWINGS

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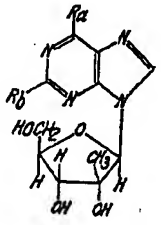
COMPLETE SPECIFICATION

Nucleosides and their Preparation

We, MERCK & CO. INC., a corporation duly organised and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement: —

This invention relates to nucleosides.

The novel compounds of the present invention have the following structural formula:



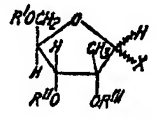
I

in which each of R_a and R_b, which may be the same or different, is a hydrogen or halogen atom or a hydroxy, C_{1-5} alkyl, amino, C_{1-5} alkylamino, di(C_{1-5} alkyl)amino, mercapto or C_{1-5} alkylthio radical.

Compounds of the present invention may be used in the preparation of various 2'-methyl nucleotides, which may be useful in the study of nucleic acid metabolism, by their reaction with phosphorus compounds.

Typical values of R_a and R_b, apart from those specifically mentioned above, are methyl, ethyl, propyl, methylamino, dimethylamino, ethylamino, diethylamino, propylamino, dipropylamino, chlorine, bromine, methylthio, methylthio and propylthio.

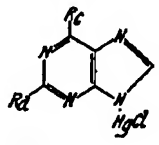
The compounds of the present invention are prepared in general by a two-step process. The first step in this process, Step A, is carried out by treating a 2,3,5-tri-O-acyl-2-methyl-*D*-ribofuranosyl halide of the following formula:



II

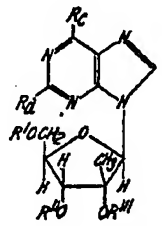
[*Pri*

with a chloromercuri-2,6-substituted purine of the formula:



III

in a solvent to form 9-(2,3,5-tri-O-acyl-2-methyl-*D*-ribofuranosyl)-2,6-substituted purine intermediates of the formula:



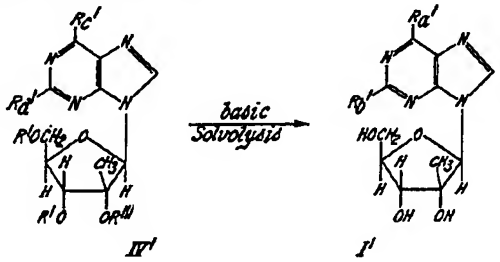
IV

in which each of R_c and R_d is halogen, hydrogen, hydroxy, C_{1-5} alkyl, acylamino or acyl C_{1-5} alkylamino, each of R', R'' and R''', which may be the same or different, is an acyl group and X is a halogen atom. It is preferred that essentially stoichiometric amounts of the reactants be used. The reaction is preferably carried out in a temperature range of from 25°C to 150°C, particularly 100°C to 140°C, for a period of time sufficient to complete the reaction. This time is usually from 15 minutes to 5 hours; the higher the temperature, the quicker the reaction. The selection of the solvent is not important as long as it is an inert solvent and that it boils in a range of about 25°C to 150°C. Examples of such solvents are benzene, dibutyl ether, cyclohexane, toluene and xylene, preferably toluene and xylene.

The 2,3,5-tri-O-acyl-2-methyl-*D*-ribofuranosyl halides used as starting materials are claimed in and may be prepared by processes described and claimed in the specification of our copending application No. 51812/69 (1,187,825). These processes comprise acylating 2-C-methyl-*D*-ribono-γ-lactone to form 2,3,5-tri-O-acyl-2-C-methyl-*D*-ribono-γ-lactone, which is reduced with a dialkyl borane to produce 2,3,5-tri-O-acyl-2-C-methyl-(α,β)-*D*-ribofuranose, which is further acylated to form 1,2,3,5-tetra-O-acyl-2-C-methyl (α,β)-*D*-ribofuranose and converted into the ribofuranosyl halide by a halogenation replacement reaction in a suitable solvent.

Those compounds of the present invention of Formula I', in which each of $R_{a'}$ $R_{b'}$ is a hydrogen or halogen atom or a hydroxy, C_{1-5} alkyl, amino, C_{1-5} alkylamino or di(C_{1-5} alkyl)amino radical, are prepared by basic solvolysis of the intermediate 9-(2,3,5-tri-O-acyl-2-methyl-*D*-ribofuranosyl)-2,6-substituted purines (Formula IV').

This reaction is illustrated as follows:



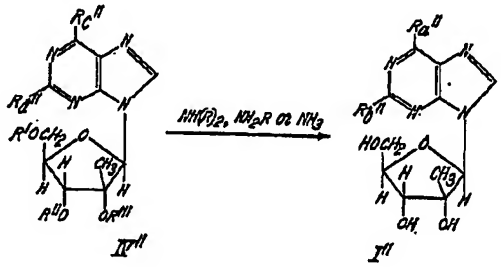
IV' I'

where in Formula IV' each of $R_{c'}$ and $R_{d'}$ is a hydrogen or halogen atom or a hydroxy, C_{1-5} alkyl, acylamino or acyl(C_{1-5} alkyl)amino radical and in Formula I' each of $R_{a'}$ and $R_{b'}$ is a hydrogen or halogen atom or a C_{1-3} alkyl, hydroxy, amino or C_{1-5} alkylamino radical.

The solvolysis reaction is carried out in the presence of a basic catalyst in an appropriate solvent, preferably in a temperature range of from 5°C to 150°C, particularly 65°C to 90°C, in a reaction time of from 15 minutes to 5 hours. The length of reaction time is dependent upon the temperature, the catalyst and solvent used. Examples of basic catalysts are alkali metal and alkaline-earth metal inorganic bases and their corresponding alkoxides, solutions of ammonia, amines and substituted amines. Suitable solvents are C_{1-4} alcohols, preferably methanol.

In another aspect of the present invention, those compounds of Formula I'', in which one or both of R_a'' and R_b'' is an amino, C_{1-5} alkylamino or di(C_{1-5} alkyl)amino radical, are prepared by an aminolysis reaction of those intermediate 9-(2,3,5-tri-O-acyl-2-methyl-*D*-ribofuranosyl)-2,6-substituted purines in which the 2 and/or 6 purine position is substituted with a halogen, designated IV''.

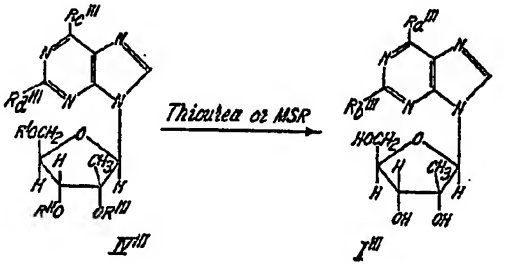
The reaction is illustrated as follows:



where at least one of R_c'' and R_d'' is a halogen atom, R in the aminolysis reagents is a C_{1-5} alkyl radical and one of R_a'' and R_b'' in Formula I'' is amino, C_{1-5} alkylamino or di(C_{1-5} alkyl)amino.

The aminolysis reaction is carried out in the presence of ammonia, a C_{1-5} alkylamine or a di(C_{1-5} alkyl)amine, preferably at a temperature from 25°C to 150°C and preferably 85°C to 100°C in a reaction time of from 15 minutes to 5 hours. Examples of amines are methylamine, dimethylamine, ethylamine, diethylamine, propylamine and dipropylamine.

In another aspect of the present invention the compounds of Formula I''', in which one or both of R_a''' and R_b''' is or are mercapto or C_{1-5} alkylthio are prepared by a mercaptolysis reaction, the preferred reagents being thiourea and thiolates of formula MSR, where R is C_{1-5} alkyl and M is an equivalent of a metal, preferably an alkali metal or an alkaline-earth metal, although any mercaptolysing agent capable of introducing a mercapto or C_{1-5} alkylthio group may be used. In the reaction scheme that follows, one or both of R_c''' and R_d''' in Formula IV''' is a halogen atom, and each of R', R'' and R''' is acyl:



When the mercaptolysis reactant is thiourea the acyl blocking groups R', R'' and R''' are not removed and the resulting intermediate must be subjected to basic solvolysis in order to obtain the compounds of the present invention, Compound I'''.

The mercaptolysis reaction is carried out in the presence of thiourea or a metal salt of a C_{1-5} alkylthiol, preferably at a temperature of from 25°C to 150°C, particularly 65°C to 90°C, in a reaction time of from about 15 minutes to about 5 hours. Preferred are the alkali metal and alkaline-earth metal salts of alkylthiols, e.g. sodium methanethiolate, sodium ethanethiolate, sodium isopropanethiolate, potassium methanethiolate and calcium methanethiolate.

Representative of the novel compounds of the present invention are
9-(2-methyl-*D*-ribofuranosyl)-2-methylpurine
9-(2-methyl-*D*-ribofuranosyl)-6-methylpurine
9-(2-methyl-*D*-ribofuranosyl)-2,6-dimethylpurine
9-(2-methyl-*D*-ribofuranosyl)-2-ethylpurine
9-(2-methyl-*D*-ribofuranosyl)-6-ethylpurine
9-(2-methyl-*D*-ribofuranosyl)-2,6-diethylpurine
9-(2-methyl-*D*-ribofuranosyl)-2-propylpurine
9-(2-methyl-*D*-ribofuranosyl)-6-propylpurine
9-(2-methyl-*D*-ribofuranosyl)-2,6-dipropylpurine
9-(2-methyl-*D*-ribofuranosyl)-2-aminopurine
9-(2-methyl-*D*-ribofuranosyl)-6-aminopurine
9-(2-methyl-*D*-ribofuranosyl)-2,6-diaminopurine
9-(2-methyl-*D*-ribofuranosyl)-2-methylaminopurine
9-(2-methyl-*D*-ribofuranosyl)-6-methylaminopurine
9-(2-methyl-*D*-ribofuranosyl)-2,6-dimethylaminopurine
9-(2-methyl-*D*-ribofuranosyl)-2-ethylaminopurine
9-(2-methyl-*D*-ribofuranosyl)-6-ethylaminopurine
9-(2-methyl-*D*-ribofuranosyl)-2,6-diethylaminopurine
9-(2-methyl-*D*-ribofuranosyl)-2-hydroxypurine
9-(2-methyl-*D*-ribofuranosyl)-6-hydroxypurine
9-(2-methyl-*D*-ribofuranosyl)-2,6-dihydroxypurine
9-(2-methyl-*D*-ribofuranosyl)-2-methyl-6-aminopurine
9-(2-methyl-*D*-ribofuranosyl)-2-methyl-6-methylaminopurine
9-(2-methyl-*D*-ribofuranosyl)-2-methylamino-6-methylpurine
9-(2-methyl-*D*-ribofuranosyl)-2-amino-6-methylaminopurine
9-(2-methyl-*D*-ribofuranosyl)-2-methyl-6-hydroxypurine
9-(2-methyl-*D*-ribofuranosyl)-2-hydroxy-6-methylpurine
9-(2-methyl-*D*-ribofuranosyl)-2-amino-6-hydroxypurine
9-(2-methyl-*D*-ribofuranosyl)-2-hydroxy-6-aminopurine
9-(2-methyl-*D*-ribofuranosyl)-2-methylamino-6-hydroxypurine
9-(2-methyl-*D*-ribofuranosyl)-2-hydroxy-6-methylaminopurine
9-(2-methyl-*D*-ribofuranosyl)-2-dimethylaminopurine
9-(2-methyl-*D*-ribofuranosyl)-6-dimethylaminopurine
9-(2-methyl-*D*-ribofuranosyl)-2-methylamino-6-dimethylaminopurine
9-(2-methyl-*D*-ribofuranosyl)-2-mercaptopurine
9-(2-methyl-*D*-ribofuranosyl)-6-mercaptopurine
9-(2-methyl-*D*-ribofuranosyl)-2,6-dimercaptopurine
9-(2-methyl-*D*-ribofuranosyl)-2-methyl-6-mercaptopurine
9-(2-methyl-*D*-ribofuranosyl)-6-methyl-6-mercaptopurine
9-(2-methyl-*D*-ribofuranosyl)-2-mercapto-6-methylmercaptopurine
9-(2-methyl-*D*-ribofuranosyl)-2,6-dichloropurine
9-(2-methyl-*D*-ribofuranosyl)-2-chloropurine
9-(2-methyl-*D*-ribofuranosyl)-2-bromopurine
9-(2-methyl-*D*-ribofuranosyl)-6-bromopurine
9-(2-methyl-*D*-ribofuranosyl)-6-chloropurine
9-(2-methyl-*D*-ribofuranosyl)-2,6-dibromopurine

Compounds of the present invention have a variety of valuable uses and have been found capable of inhibiting ribonucleic acid (RNA) synthesis, for example, acid insoluble RNA synthesis, in Ehrlich ascites cells and KB cells. In invitro tests, the growth of KB cells has been shown to be markedly suppressed by compounds of the present invention as is the incorporation of hypoxanthine into acid insoluble RNA. Compounds of the present invention are therefore useful as antimetabolites, as cell growth inhibitors and for the study of metabolism systems and have also been shown to possess favourable cytotoxicity characteristics considered with their cell growth depression.

Compounds of the present invention may also be converted to nucleotides by treatment with phosphoric acid derivatives in accordance with known techniques. As such, they are useful in a formulation of media for selective culturing of animal tissue cells. These nucleotides may also be useful in the study of nucleic acid metabolism.

The following examples illustrate the compounds of the present invention. Examples 1—7 are concerned with the preparation of intermediate compounds, Examples 1 and 1A being examples of the invention claimed in the specification of

our copending application No. 51812/69 (1,187,825). In the Examples all parts are by weight and the word 'Dowex' is a trade mark.

EXAMPLE 1

Preparation of 2,3,5-Tri-O-benzoyl-2-methyl-*D*-ribofuranosyl chloride

This example shows the synthesis of a novel starting material used in the preparation of the compound of the present invention.

A solution of 5 g. (30.8 millimoles) of 2-C-methyl-D-ribono-γ-lactone in 100 ml. of dry pyridine is cooled to about 5°C. and treated with 17 ml. of benzoyl chloride. The mixture is heated to 65—70°C. for 4 hours and kept at room temperature for 16 hours. The reaction mixture is stirred with 2 ml. of water for 25 minutes to decompose unreacted benzoyl chloride, and the pyridine is removed at reduced pressure. The thick residue is dissolved in 100 ml. of chloroform and the chloroform solution is washed with three 50-ml. portions of 10 percent hydrochloric acid, two 50-ml. portions of 10 percent sodium bicarbonate and two 50-ml. portions of water. The dried chloroform solution is concentrated and the residue is dissolved in ether. The ethereal solution is concentrated to 250 ml. and after being cooled to 5°C. for several hours gives 8.8 g. (60%) of 2,3,5-tri-O-benzoyl-2-C-methyl-*D*-ribo-γ-lactone, m.p. 138—140°C.

A solution of 7 g. (14.7 mmole) of 2,3,5-tri-O-benzoyl-2-C-methyl-*D*-ribono-γ-lactone in 30 ml. of dry tetrahydrofuran is cooled, under nitrogen, and treated with 58.8 ml. of 1 M disecondaryisoamylborane. The reaction solution is kept at room temperature for 16 hours. After the careful addition of 6 ml. of water, the mixture is refluxed for 0.5 hour. The mixture is cooled to about 5°C, and 11.5 ml. of 30% hydrogen peroxide is added while the pH is maintained between 7 and 8 through the addition of about 7 ml. of 3N sodium hydroxide. The mixture is extracted with six 50-ml. portions of chloroform and the extracts are washed with several portions of water. The remaining peroxides are removed by washing the chloroform solution with 30% ferrous sulfate. Concentration of the chloroform layer gives 7.8 g. of crude product as a syrup. The product is purified by chromatography on 200 g. of silica gel in a mixture of benzene and ethyl acetate (4:1). From the column, fractions are obtained which contain 2.5 g. (37%) of 2,3,5-tri-O-benzoyl-2-C-methyl-(α and β)-*D*-ribofuranose.

A solution of 4.2 g. (8.8 millimoles) of 2,3,5-tri-O-benzoyl-2-C-methyl-(α,β)-*D*-ribofuranose (containing a small amount of 3,5-di-O-benzoyl-2-C-methyl-(α,β)-*D*-ribofuranose) in 80 ml. of dry pyridine is cooled and treated with 8.0 ml. (68 millimoles) of benzoyl chloride. The mixture is heated at 90°C. for 4 hours and cooled to about 5°C. A small amount of water is added and the mixture is stirred for 0.5 hour to decompose excess of benzoyl chloride. The reaction mixture is concentrated and the residue is dissolved in chloroform. The chloroform solution is washed with three 50-ml. portions of 10% hydrochloric acid, three 50-ml. portions of saturated sodium bicarbonate and three 50-ml. portions of water. The dried chloroform layer is concentrated to 5.1 g. of an oil. Addition of 50 ml. of ether gives 2.16 g. (42%) of 1,2,3,5-tetra-O-benzoyl-2-C-methyl-β-*D*-ribofuranose, m.p. 155—156°C. Concentration of the filtrate gives 2.9 g. (57%) of essentially pure 1,2,3,5-tetra-O-benzoyl-2-C-methyl-α-*D*-ribofuranose as an oil.

To 100 ml. of a saturated solution of hydrogen chloride in ether is added 2 ml. of acetyl chloride and 1.5 g. (2.6 millimoles) of 1,2,3,5-tetra-O-benzoyl-2-C-methyl-β-*D*-ribofuranose. The solution is kept at room temperature for 2 hours and the ether is removed at reduced pressure. Five 25-ml. portions of dry toluene are successively removed at reduced pressure from the residue. The residue is dissolved in dry ether and quickly washed with cold saturated sodium bicarbonate and finally with cold water. After being dried the ethereal solution is concentrated and a residue of 2,3,5-tri-O-benzoyl-2-C-methyl-β-*D*-ribofuranosyl chloride is obtained.

EXAMPLE 1A

Preparation of 2,3,5-Tri-O-benzoyl-2-methyl-*D*-ribofuranosyl Bromide

A solution of 1.5 g. (2.6 millimoles) of 1,2,3,5-tetra-O-benzoyl-2-C-methyl-α-*D*-ribofuranose as prepared in Example 1 in 7.5 ml. of acetic acid is treated with 0.25 ml. of acetyl bromide and 7.5 ml. of a 32% (w/w) solution of hydrogen bromide in acetic acid. The mixture is kept at 25°C. for 24 hours. The mixture is concentrated and a portion of dry toluene is distilled, at reduced pressure, from the residue to remove excess hydrogen bromide and acetic acid. The residue is dissolved in dry ether and quickly washed with cold saturated sodium bicarbonate and finally with cold water. After being dried the ethereal solution is concentrated and a residue of 2,3,5-tri-O-benzoyl-2-C-methyl-β-*D*-ribofuranosyl bromide is obtained.

EXAMPLE 2

Preparation of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-2-acetamido-6-hydroxypurine

About 25 ml. of xylene is distilled from a suspension of 5.95 grams (0.014 mole) of chloromercuri 2-acetamido-6-hydroxypurine in 175 ml. of xylene to remove the last traces of water. The suspension is cooled to 25°C. and 2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl chloride prepared from 8.1 grams (0.014 mole) of 1,2,3,5-tetra-O-benzoyl-2-methyl-*D*-ribofuranose in 25 ml. of dry xylene is added. The mixture is stirred and heated at a temperature of from about 50°C. to about 100°C. The solid changes from a granular form to flocculent. After being refluxed for one hour, the hot mixture is filtered, which removes the undissolved solids. Leaching the solids with three 50-ml. portions of boiling chloroform removes additional soluble product and leaves insoluble starting chloromercuri derivatives and inorganic salts. The original filtrate is diluted with two volumes of petroleum ether and the solid which separates is dissolved in the chloroform solution obtained above. The chloroform solution plus an additional 100 ml. is washed with two 75 ml. portions of 30% potassium iodide solution and two 75-ml. portions of water. The dry chloroform layer is concentrated and 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-2-amino-6-hydroxypurine is obtained.

EXAMPLE 3

Preparation of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-N-methylbenzamidopurine

About 150 ml. of xylene is distilled from a suspension of 9.5 grams (19.5 millimoles) of chloromercuri 6-N-methylbenzamidopurine in 500 ml. of xylene. The mixture is cooled and a solution of 2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl chloride (from 8.2 grams [14.1 millimoles] of 1,2,3,5-tetra-O-benzoyl-2-methyl-*D*-ribofuranose) in 50 ml. of dry xylene is added. The reaction mixture is stirred and refluxed for 30 minutes. The hot mixture is filtered and 3 grams of unreacted starting chloromercuri purine is recovered. The filtrate is concentrated to dryness and the residual oil in 300 ml. of chloroform is washed with two 80-ml. portions of 30% potassium iodide solution and two 80-ml. portions of water. The residual oil obtained after removal of the chloroform is chromatographed on a short column of 140 grams of acid-washed alumina in 9 to 1 benzene-chloroform. Fractions are combined and concentrated giving 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-N-methylbenzamidopurine.

EXAMPLE 4

Preparation of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-chloropurine

About 100 ml. of xylene is distilled from a suspension of 6.55 grams (16.8 millimoles) of chloromercuri-6-chloropurine in 460 ml. of xylene in order to remove the last traces of water. A solution of 9.05 grams (16.8 millimoles) of 2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl bromide in 40 ml. of dry xylene is added to the stirred suspension at 25°C. The mixture is refluxed for 2 hours. The hot mixture is filtered to remove insoluble material. The filtrate is concentrated to 150 ml. and diluted with 300 ml. of petroleum ether. The mixture is kept at 5°C. for one hour and filtered. The solid is washed with three 20-ml. portions of petroleum ether and dried. The crude product is dissolved in 300 ml. of hot chloroform and washed with two 80-ml. portions of 30% potassium iodide solution and two 80-ml. portions of water. The dried ($MgSO_4$) chloroform layer is concentrated, and 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-chloropurine is obtained. The product is purified by chromatography on a short alumina column in chloroform.

EXAMPLE 5

Preparation of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-2,6-dibenzamidopurine

About 100 ml. of xylene is distilled from suspension of 5.01 grams (8.43 millimoles) of chloromercuri, 2,6-dibenzamido purine in 370 ml. of xylene to remove last traces of water. The suspension is cooled to room temperature in a solution of 4.55 grams (8.43 millimoles) of 2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl bromide in 37 ml. of dry xylene is added while the suspension is being stirred. The mixture is refluxed for 2 hours and filtered hot which removes insoluble material. The filtrate is diluted with 400 ml. of petroleum ether and cooled in an ice bath. The solid is removed and dried. The product is obtained as a complex with mercuric halide. The product is dissolved in 100 ml. of chloroform and washed with two 40-ml. portions

of 30% potassium iodide solution and two 40-ml. portions of water. The dried ($MgSO_4$) chloroform solution is concentrated at reduced pressure to give 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-2,6-dibenzamidopurine.

Example 6

Preparation of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-methylpurine

A suspension of 3.7 grams (10 millimoles) of chloromercuri 6-methylpurine [Davoll and Lowy, J. Am. Chem. Soc. 73 1650 (1951)] in 200 ml. of xylene is dried by distilling about 50 ml. of xylene. The cooled suspension is treated with 4.94 grams (10 millimoles) of 2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl chloride dissolved in 30 ml. of dry xylene. The mixture is stirred and refluxed for 2 hours and it is filtered to remove insoluble material. The filtrate is diluted with 4 volumes of petroleum ether and, after being cooled for about 2 hours in an ice bath, the mixture is filtered. The solid is dissolved in 200 ml. of chloroform and washed with two 30-ml. portions of 20% aqueous potassium iodide solution. The chloroform layer is dried (anhydrous $MgSO_4$) and concentrated to a residue of amorphous 9-(2,3,5-tri-O-benzoyl-2-methyl-ribofuranosyl)-6-methylpurine.

Example 7

Preparation of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-benzamidopurine

A suspension of 2.82 grams (5.95 millimoles) of finely ground chloromercuri 6-benzamidopurine in 200 ml. of xylene is dried by distilling 100 ml. of xylene. The mixture is cooled and a solution of 2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl chloride [made from 3.45 grams (5.95 millimoles) of 1,2,3,5-tetra-O-benzoyl-2-methyl-*D*-ribofuranose] in 30 ml. of dry xylene is added. The mixture is stirred and refluxed for 80 minutes. The hot mixture is filtered and the solid is washed with 25 ml. of hot xylene. The filtrate and washings are diluted with 400 ml. of petroleum ether, and after being kept at 5°C. for 20 hours, the mixture is filtered. The solid is dissolved in 300 ml. of chloroform and the solution is washed with two 20-ml. portions of 30% potassium iodide solution and two 20-ml. portions of water. Concentration of the dried chloroform layer gives amorphous product which is chromatographed on 70 grams of alumina in ethyl acetate-chloroform (1:4). Fractions showing only one zone (R_f 0.65) after thin layer chromatography on alumina in ethyl acetate-chloroform (1:4) are combined and concentration of the solvent gives 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-benzamidopurine as an amorphous solid.

Example 8

Preparation of 9-(2-Methyl-*D*-ribofuranosyl)-6-dimethylaminopurine

A suspension of 1.0 gram (1.57 millimole) of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-chloropurine as prepared in Example 4 in 25 ml. of methanol containing 6.5 grams of dimethylamine is heated for 10 hours in a sealed tube at 100°C. The solution is concentrated at reduced pressure and the residue is dissolved in 25 ml. of water. The water solution is washed with five 8-ml. portions of benzene and then treated with 2 grams of *Dowex* II-X8 which is a strongly basic anion-exchange resin having a styrene divinyl benzene polymer matrix and containing quaternary ammonium groups. It has an average particle size in the range of 50—100 mesh. It is manufactured by the Dow Chemical Co. of Midland, Michigan (see Page 1576, 7th Ed., Merck Index, Merck & Co., Inc., Rahway, N.J. The resin is filtered and washed with three 25-ml. portions of water. The filtrate is concentrated to dryness and 9-(2-methyl-*D*-ribofuranosyl)-6-dimethylaminopurine is obtained.

Example 9

Preparation of 9-(2-methyl-*D*-ribofuranosyl)-2,6-diaminopurine

A mixture of 1.2 grams (1.37 millimoles) of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-2,6-dibenzamidopurine as prepared in Example 5 in 12 ml. of dry methanol is treated with a solution of 97 mg. of (4.2 millimoles) of sodium in 12 ml. of methanol. The mixture is refluxed for 3 hours and the resultant solution is concentrated at reduced pressure. The residue is dissolved in 24 ml. of water and the pH is adjusted to about 6.5. The aqueous solution is extracted with five 10-ml. portions of chloroform to remove methyl benzoate and concentrated at reduced pressure to a residue containing 9-(2-methyl-*D*-ribofuranosyl)-2,6-diaminopurine.

Example 10

Preparation of 9-(2-methyl-*D*-ribofuranosyl)-purine-6-thiol

A suspension of 1.25 grams (1.96 millimoles) of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-chloropurine, prepared as in Example 4, and 307 mg. (4.0 millimoles) of thiourea in 3 ml. of ethanol is refluxed for 40 mniutes. After 5 minutes a clear colorless solution is obtained which becomes yellow in 15 minutes and shortly thereafter colorless crystals of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-purine-6-thiol crystallize out of solution.

A suspension of 400 mg. (0.64 millimoles) of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-purine-6-thiol in 3.5 ml. of dry methanol is treated with a solution made from 19.5 mg. of sodium and 3.5 ml. of dry methanol is added. Complete solution occurs immediately. The solution is refluxed for three hours. The solution is concentrated by distillation at reduced pressure and the residue is dissolved in 6 ml. of water and the pH of the solution is adjusted to 9 with acetic acid and the aqueous mixture is extracted with four 1.5-ml. portions of methylene chloride. The aqueous layer is concentrated by distillation to a volume of 4 ml. and the pH is adjusted to 4 with acetic acid. The concentration of the solution gives a residue containing 9-(2-methyl-*D*-ribofuranosyl)-purine-6-thiol.

Example 11

Preparation of 9-(2-methyl-*D*-ribofuranosyl)-6-methylaminopurine

A mixture of 1 gram (1.6 millimoles) of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-chloropurine and 8 grams of methylamine in 25 grams of dry methanol is heated for 10 hours at 100°C. in a sealed tube. The solution is concentrated to dryness at reduced pressure and the residue is dissolved in 25 ml. of water. The aqueous solution is washed with two 5-ml. portions of benzene. The aqueous layer is stirred for 2.5 hours with 3.5 grams of moist *Dowex* II-X8 (see Example 8), during which time the pH of the solution rises from 7 to 9. The resin is removed and washed with three 15-ml. portions of water. The filtrate and washings are concentrated to a residue containing 9-(2-methyl-*D*-ribofuranosyl)-6-methylaminopurine.

Example 12

Preparation of 9-(2-methyl-*D*-ribofuranosyl) purine

A solution of 1 gram (1.6 millimoles) of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-chloropurine in 17 ml. of dioxane with 80 mg. (2.0 millimoles) of magnesium oxide and 0.5 grams of 5% palladium on charcoal catalyst is shaken for 98 hours in an atmosphere of hydrogen at 25°C. The mixture is filtered and concentrated by distillation at reduced pressure to a residue containing 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)purine.

A solution of 400 mg. (0.69 millimoles) 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)purine in 8 ml. of dry methanol is treated with a solution made from 23 mg. (1 mg. atom) of sodium and 8 ml. of dry methanol. The pale yellow solution is refluxed for 3 hours and concentrated to dryness at reduced pressure. The residue is dissolved in 15 ml. of water and the pH is adjusted to 6.5 with acetic acid. The solution is extracted with four 5-ml. portions of chloroform and the aqueous phase is concentrated to dryness at reduced pressure to a residue containing 9-(2-methyl-*D*-ribofuranosyl)purine.

A suspension of 800 mg. (1.2 millimoles) of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-2-acetamido-6-hydroxypurine in 8 ml. of dry methanol is treated with a solution made from 105 mg. (4.5 mg. atom) of sodium and 8 ml. of dry methanol and the mixture is refluxed for two hours. The mixture is concentrated to dryness. The residue is dissolved in 35 ml. of water and the pH is adjusted to 7 by the addition of acetic acid. The clear solution is washed with three 8-ml. portions of chloroform and the aqueous layer is concentrated to a residue of 9-(2-methyl-*D*-ribofuranosyl)guanine.

Example 13

Preparation of 9-(2-methyl-*D*-ribofuranosyl)-6-chloropurine

A solution of 479 mg. (0.98 millimole) of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-chloropurine, prepared as in Example 4, in 20 ml. of cold methanol containing 2 grams of anhydrous ammonia is kept at 5°C. for 20 hours. The solution is concentrated at reduced pressure and at a temperature of less than 20°C. The residue is recrystallized from methanol to give 9-(2-methyl-*D*-ribofuranosyl)-6-chloropurine.

Example 14

Preparation of 9-(2-methyl-*D*-ribofuranosyl)-6-methylaminopurine

About 150 ml. of xylene is distilled from a suspension of 9.5 grams (19.5 millimoles) of chloromercuri-6-(N-methylbenzamido) purine in 500 ml. of xylene. The

mixture is cooled and a solution of 2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl chloride [from 8.2 grams (14.1 millimole) of 1,2,3,5-tetra-O-benzoyl-2-methyl-β-D-ribofuranose] in 50 ml. of dry xylene is added. The reaction mixture is stirred and refluxed for 30 minutes. The hot mixture is filtered and 3 grams of unreacted starting chloromercuri purine is recovered. The filtrate is concentrated to dryness and the residual oil in 300 ml. of chloroform is washed with two 100-ml. portions of 30% potassium iodide and two 100-ml. portions of water. The residual oil obtained after removal of the chloroform is chromatographed on a short column of 140 grams of acid-washed alumina in benzene-chloroform (1:9). Fractions containing only product are combined and concentrated giving 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-(N-methylbenzamido)purine as a glass.

A suspension of 3.9 grams (5.45 millimoles) of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-(N-methylbenzamido)purine in 40 ml. of dry methanol is treated with a solution made from 175 mg. (7.6 mg. atom) of sodium in 40 ml. of dry methanol and the solution is refluxed for 3.5 hours. The methanol is removed and the solution of the residue in 76 ml. of water is neutralized (pH 7.0) with acetic acid and washed with three 10-ml. portions of chloroform. The aqueous layer is concentrated by distillation to a residue of 9-(2-methyl-*D*-ribofuranosyl)-6-methylaminopurine.

EXAMPLE 15

Preparation of 9-(2-methyl-*D*-ribofuranosyl)-6-ethylaminopurine

A solution of 2.0 grams (3.2 millimoles) of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-chloropurine prepared as in Example 4, in 30 ml. of ethanol containing 12 ml. of ethyl amine is heated in a sealed tube at 100°C. for 10 hours. After removing the solvent, the residue is dissolved in 60 ml. of water and extracted with three 15-ml. portions of ether. The aqueous layer (pH 6.5) is stirred for 1 hour with 2.5 grams of *Dowex* II-X8 (see Example 8). The resin is removed and washed with four 10-ml. portions of water. The combined filtrate and washings are concentrated to a residue of 9-(2-methyl-*D*-ribofuranosyl)-6-ethylaminopurine.

EXAMPLE 16

Preparation of 9-(2-methyl-*D*-ribofuranosyl)-6-methylthiopurine

A boiling mixture of 605 mg. (1.9 millimoles) of 9-(2-methyl-*D*-ribofuranosyl)-6-chloropurine, prepared as in Example 14, in 30 ml. of anhydrous methanol is treated with a solution prepared by saturating 20 ml. of 0.1 N sodium methoxide in methanol with methyl mercaptan. After being refluxed for about 30 minutes the solution is cooled and concentrated to dryness. The residue is dissolved in hot water and on cooling, 9-(2-methyl-*D*-ribofuranosyl)-6-methylthiopurine separates.

EXAMPLE 17

Preparation of 9-(2-methyl-*D*-ribofuranosyl)-6-methylpurine

A mixture of 590 mg. (0.98 millimole) of 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-methylpurine, prepared as in Example 6, and 50 ml. of dry methanol is treated with a solution prepared from 23 mg. (1 mg. atom) of sodium and 10 ml. of dry methanol. The mixture is refluxed for 4 hours and concentrated to dryness. The residue is dissolved in 30 ml. of water and neutralized (pH 7) with acetic acid. When the aqueous layer is concentrated to a small volume and cooled, 9-(2-methyl-*D*-ribofuranosyl)-6-methylpurine precipitates.

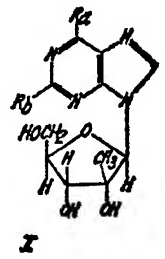
EXAMPLE 18

Preparation of 2'-methyladenosine

A mixture of 1.48 grams (2.08 millimoles) of 9-(2,3,5-tri-O-benzoyl-2-methyl-β-D-ribofuranosyl)-6-benzamidopurine prepared as in Example 7 and 15 ml. of dry methanol is treated with a solution of sodium methoxide prepared from 70 mg. (3 millimoles) of sodium and 5 ml. of methanol. After the mixture has been refluxed for 45 minutes, it is concentrated and the residue is dissolved in 50 ml. of water. The pH is adjusted to 6.8 with a few drops of acetic acid. The solution is extracted with three 20-ml. portions of ether and the aqueous layer is filtered and concentrated to about 5 ml. The solid obtained is recrystallized from 7 ml. of warm water, giving 360 mg. (59%) of 2'-methyladenosine.

WHAT WE CLAIM IS:—

1. Compounds of the formula:



I

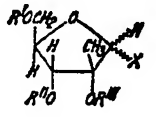
in which each of R_a and R_b, which are the same as or different from one another, is a hydrogen or halogen atom or a hydroxy, C_{1-5} alkyl, amino, C_{1-5} alkylamino, di(C_{1-5} alkyl)amino, mercapto or C_{1-5} alkylthio radical.

2. 2'-Methyladenosine.

3. 9-(2-Methyl-D-ribofuranosyl)guanine.

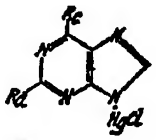
4. 9-(2-Methyl-D-ribofuranosyl)-purine-6-thiol.

5. The process that comprises, in a first step, treating a compound of the formula:



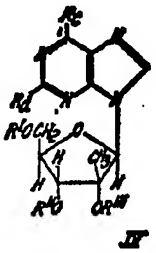
II

in which R', R'' and R''' are the same or different acyl groups and X is a halogen atom with a compound having the formula:



III

in which each of R_c and R_d, which are the same as or different from one another, is a halogen or hydrogen atom or a hydroxy, C_{1-6} alkyl, acylamino or acyl-(C_{1-5} alkyl)-amino radical, in a solvent to form a compound of the formula:



IV

and, in a second step, subjecting the latter to basic solvolysis to produce a compound as claimed in claim 1 in which each of R_a and R_b is a halogen or hydrogen atom or a hydroxy, C_{1-5} alkyl, amino or C_{1-5} alkylamino radical; or, but only when R_c and/or R_d in Compound IV is halogen, to aminolysis with ammonia, a C_{1-5} alkylamine or a di(C_{1-5} alkyl)amine to produce a compound as claimed in claim 1 in which R_a and/or R_b is amino, C_{1-5} alkylamino or di(C_{1-5} alkyl)amino; or, but only when R_c and/or R_d in Compound IV is halogen, to mercaptolysis to produce a compound as claimed in claim 1 in which R_a and/or R_b is mercapto or C_{1-5} alkylthio, the mercaptolysis being followed by basic solvolysis when the mercaptolysing agent is thiourea.

6. A process according to claim 5, in which the first step is carried out at a temperature of 25°C to 150°C for a period of time from 15 minutes to 5 hours; the basic solvolysis is carried out at a temperature of from 5°C to 150°C for a period of time from 15 minutes to 5 hours; the aminolysis is carried out at a temperature of from 25°C to 150°C for a period of time from 15 minutes to 5 hours; and the mercaptolysis is carried out at a temperature of from 65°C to 90°C for a period of time from 15 minutes to 5 hours.

7. A process according to claim 5 or 6, in which the second step is solvolysis and the solvolysing agent is an alkali metal or alkaline-earth metal hydroxide or alkoxide, ammonia or an amine in a C_{1-4} alcohol.

8. A process according to claim 5 or 6, in which the second step is mercaptolysis and the mercaptolysing agent is thiourea or a salt of a C_{1-5} alkylthiol.

9. A process according to claim 8, in which the salt is an alkali metal or alkaline-earth metal salt of a C_{1-5} alkyl mercaptan.

10. The process that comprises treating 2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl chloride with chloromercuri (6-benzamidopurine in the presence of xylene at reflux for 80 minutes and then held at 5°C for 20 hours to produce 9-(2,3,5-tri-O-benzoyl-2′-methyl-*D*-ribofuranosyl)-6-benzamidopurine, refluxing said 9-(2,3,5-tri-O-benzoyl-2-methyl-*D*-ribofuranosyl)-6-benzamidopurine with sodium methoxide for 45 minutes in methanol thereby forming 2′-methyladenosine.

11. A process that produces a compound as claimed in claim 1, substantially as hereinbefore described in any one of Examples 8—18.

12. A compound as claimed in claim 1 when prepared by a process according to any one of claims 5—11 or an obvious chemical equivalent of such a process.

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